

Amendments to the Specification

At the indicated page and line number, please replace the existing sections or paragraphs with the following sections or paragraphs:

(Page 11, line 28 through page 12, line 11)

A gene of a *Streptomyces* species or strain, which gene is a "homologue" of or is "homologous" to the *scbA* gene of *S. coelicolor*, may be the gene which shows greatest deduced amino acid sequence identity to *scbA* of all genes of said species or strain; alternatively or additionally, it may be a gene which is capable of specific hybridisation with the amplification product obtained using the primers oligo1 (5'-GACCACGT(CG)CC(CG)GGCATG; SEQ ID NO: 1) and oligo2 (5'-GTCCTG(CG)TGGCC(CG)GT(CG)AC(CG)CG(CG)AC; (SEQ ID NO: 2)) to amplify total DNA of said species or strain (bracketed nucleotides indicate positions of degeneracy); alternatively or additionally, it may be a gene encoding a polypeptide having at least about 35% sequence identity with the deduced amino acid sequence of *scbA* as shown in Fig. 10, preferably at least about 40% (which is the homology found between *scbA* and other homologues of the *afsA* gene of *S. griseus*) more preferably about 50%, 60%, 65% (which is the homology found between *scbA* and *afsA* of *S. griseus*), 70%, 80%, 90%, or 95%.

(Page 12, lines 13 through 32)

A gene of a *Streptomyces* species or strain, which gene is a "homologue" of or is "homologous" to the *scbR* gene of *S. coelicolor*, may be the gene which shows greatest deduced amino acid sequence identity to *scbR* of all genes of said species or strain; alternatively or additionally, it may be a gene which is adjacent to and divergent from a gene which is capable of specific hybridisation with the amplification product obtained using the primers oligo1 (5'-GACCACGT(CG)CC(CG)GGCATG; (SEQ ID

NO: 1) and oligo2 (5'-GTCCTG(CG)TGGCC(CG)GT(CG)AC(CG)CG(CG)AC;
(SEQ ID NO: 2) to amplify total DNA of said species or strain
(bracketed nucleotides indicate positions of degeneracy);
alternatively or additionally, it may be a gene encoding a
polypeptide having at least about 35% sequence identity with
the deduced amino acid sequence of *scbR* as shown in Fig. 9,
preferably at least about 40%, more preferably about 45% (which
is the homology found between *scbR* and *arpA* of *S. griseus*),
50%, 55% (which is the homology found between *scbR* and the *FarA*
gene of *S. lavendulae*) 60%, 65%, 70%, 80%, 90%, or 95%.

(Page 19, lines 3 through 11)

Fig. 4b ScbR binding sites No.1 and No.2 from Dnase I
footprinting experiments. The protected sequences are
indicated by lines and the numbering is with respect to
the transcriptional start site of *scbA* for binding site
No.1 and *scbR* for binding site No.2. The arrows and *pscbA*,
pscbR indicate the transcriptional start site and
direction of *scbA* and *scbR*, respectively. [ScbA] (amino
acid sequence is SEQ ID NO: 14) and [ScbR] (amino acid
sequence is SEQ ID NO: 15) indicate the coding sequence
for *scbA* and *scbR*, respectively. Nucleic acid sequences
are SEQ ID NO: 12 (top) and SEQ ID NO: 13 (bottom).

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Fig. 9 Deduced amino acid sequence of ScbR (SEQ ID NO: 16).

Fig. 10 Deduced amino acid sequence of ScbA (SEQ ID NO: 17).

Fig. 11 Deduced amino acid sequence of ScbB (SEQ ID NO: 18).

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Fig. 14 Nucleic acid sequence (SEQ ID NO: 19) of region
containing *scbA*, *scbR* and *scbB*. M751 ($\Delta scbA$) is deleted

from nt position 1320 to 2021; M752 ($\Delta scbR$) is deleted from nt position 2359 to 2796 with five bases added; pIJ6134 runs from nt position 2021 to 4346; and pIJ6140 runs from nt position 1 to 3430.

(Page 34, lines 8 through 23)

The synthetic oligonucleotides oligo1;

5'-GACCACGT(CG)CC(CG)GGCATG (SEQ ID NO: 1) and oligo2;

5'-GTCCTG(CG)TGGCC(CG)GT(CG)AC(CG)CG(CG)AC (SEQ ID NO: 2)

(bracketed nt indicate positions of degeneracy) were used in the PCR (Erlich, 1989) to amplify the internal segment of *scbA* from *S. coelicolor* M145 total DNA (Fig. 2a). The reaction mixture contains: 10x reaction mixture supplied by Boehringer Mannheim, 200 μ M final concentration of four dNTPs, 5% final concentration of DMSO, 50pmol of each primer, 50ng of chromosomal DNA in a final volume of 100 μ l. After denaturation by boiling 5 min, 2.5U of Taq polymerase was added and subjected to 30 cycles of denaturation at 94°C for 50 sec, annealing at 55°C for 40 sec and extension at 72°C for 40 sec, and then incubated at 72°C for 10 min. PCR products were analyzed on a 2% w/v agarose gel electrophoresis.

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To complement M751, *scbA* coding sequence with its promoter region was amplified by PCR from *S. coelicolor* M145 cosmid GB10 DNA. Two synthetic oligonucleotides 5'-GCCAGCAGGTGGGCGACCTGAC (1796nt position; SEQ ID NO: 3) and 5'-GATCGCCCGGTCCTGCTTGGCCATG (3055nt position; SEQ ID NO: 4) were used. The PCR conditions were as stated above except the High Fidelity Kit (Boehringer Mannheim) was used and the PCR cycle was reduced to 20. The PCR product was purified by a Sephadex G-50 (Pharmacia) spin column then ligated to the pGEM easy vector (Promega) and transformed to JM101. The sequence of the transformant was confirmed by using the ABI automated sequencer and Big Dye dye terminator cycle sequencing kit

(Perkin Elmer).

(Page 35, line 16 through page 36, line 5)

For each S1 nuclease reaction, 30 or 40 μ g of RNA were hybridized in NaTCA buffer (Murray, 1986; Solid NaTCA(Aldrich) was dissolved to 3M in 50mM PIPES, 5mM EDTA, pH7.0) to about 0.002pmol (approximately 10⁴Cerenkov counts min 10⁻¹) of the following probes. For *scbA* the synthetic oligonulceotide 5'-TATCCAGCTGACCGGGAACGCGTC (SEQ ID NO: 5), corresponding to the region within the coding region of *scbA* was labelled with [³²P]-ATP using T4 polynucleotide kinase uniquely at the 5' end of the oligonucleotide, then used in the PCR reaction with the unlabelled oligonucleotide 5'-ATCGCCCGGTCCTGCTTGGCCATG (SEQ ID NO: 6) which corresponds to a region upstream of the *scbA* promoter region to generate a 259bp probe. For *scbR*, the synthetic oligonulceotide 5'-AAGTAGAGGGCTCCCTTGGTCA (SEQ ID NO: 7), corresponding to the region within the coding region of *scbR* was labelled with [³²P]-ATP using T4 polynucleotide kinase uniquely at the 5' end of the oligonucleotide, then used in the PCR reaction with the unlabelled oligonucleotide 5'-CAAACTACTGCTTCGGGCATG (SEQ ID NO: 8) which corresponds to a region upstream of the *scbR* promoter region to generate a 280bp probe. Both PCR reactions were done using M145 total DNA as template. For *hrdB*, the probe was made as previously described (Buttner et al., 1990). Subsequent steps were as described by Strauch et al. (1991).

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50pmol of the synthetic oligonucleotides 5'-CTGCACCCTGGTCCGGTGGACA (SEQ ID NO: 9) and 5'-ATCGCCCGGTCCTGCTTGGCCATG (SEQ ID NO: 10) were both labelled with [³²P]-ATP using T4 polynucleotide kinase uniquely at the 5' end of the oligonucleotide, then used in the PCR reaction with the unlabelled synthetic oligonucleotide corresponding to the other primer to generate a 244bp DNA fragment. The PCR

amplified fragment was further purified by Qiagen PCR purification kit. The gel retardation assay reaction mixture contains; 5x gelretardation buffer(125mM HEPES pH7.5, 20mM DTT, 10mM ATP, 20% glycerol) 200mM KCl, 0.16 μ g/ μ l calf thymus DNA, and 0 to 15 μ l of JM101 crude extract containing ScbR protein in a final volume of 12.5 to 25 μ l. The final concentration of DNA fragments used was 2.5 ng/ml. The mixture was incubated at room temperature for 10 min then 2 μ l of dye(50%(w/v) glycerol with BPB in TE) was added to the mixture and 10 μ l was loaded to a 5%(w/v) non-denaturing polyacrylamide gel buffered with TBE. SCB1 was added to the reaction mixture either prior to incubation, or after 10 min of incubation then incubated for further 10 min.

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The in-frame deletion mutant of *scbA* was constructed by digesting pIJ6136 which contains a 1.4kb flanking DNA of *scbA* in pIJ2925 (Fig. 1) with *Bam*HI and end filled using Klenow fragment and ligated with a 1.1kb *Pvu*II -*Hinc*II fragment from pIJ6111. The transformants were analysed to find the *Pvu*II -*Hinc*II fragment was inserted with the internal *Pst*I site at the *Eco*RI side of the multiple cloning site of pIJ6136 and designated pIJ6137. The *Bgl*II fragment of pIJ6137 was inserted into the *Bam*HI site of pKC 1132 (Bierman et al., 1992) to give pIJ6140 (Fig. 1). The in-frame deletion mutant of *scbR* was constructed by PCR using the High Fidelity Kit (Beoringher Mannheim) with a universal primer and

5'-CATCTGCAGCGTGATCGTGGCAGCTTGGTAG (3130nt position; SEQ ID NO: 11) primer designed to give a 1.059kb DNA fragment flanking *scbA* as described earlier. A *Pst*I site was designed into the end of this fragment to enable ligation with a *Pst*I site internal of *scbR*. pIJ6111 was used as template for the PCR reaction and the amplified product was cloned into pGEM-T vector (Promega) to give pIJ6148. The sequence of the PCR

amplified insert of pIJ6148 was confirmed by ABI automated sequencing. The *Bam*HI-*Kpn*I 3kb fragment of pIJ6111 was cloned into pBluescript SK+ (Stratagene) to give pIJ6131. The 1.059kb *Bam*HI-*Pst*I fragment was isolated from pIJ6148 and cloned into the *Bam*HI-*Pst*I digested pIJ6131 to give pIJ6152. pIJ6152 was then digested with *Kpn*I and blunt ended then further digested with *Bam*HI. This 2.48kb DNA fragment was cloned into pKC1132 digested with *Bam*HI and *Eco*RV to give pIJ6134 (Fig. 1). Both plasmids were introduced into the methylation deficient *E. coli* strain ET 12567 containing the RP4 derivative pUZ8002 (Paget et al., 1999) and transferred into *S. coelicolor* M145 by conjugation. Single-crossover exconjugants were selected on SFM containing apramycin. Three such single colonies were then taken through three rounds of non-selective growth on SFM to promote the second crossover. Spores were then plated for single colonies which were scored for apramycin sensitivity. Deletions within *scbA* and *scbR* were confirmed by PCR using primers corresponding to flanking sequences, and by Southern hybridisation. For *scbA*, nine out of 20 apramycin sensitive colonies were deleted for *scbA* while 11 had reverted to wildtype. For *scbR*, 4 out of 20 apramycin sensitive colonies were deleted for *scbR* while 16 reverted to wildtype. The *scbA* and *scbR* deletion mutants were called M751 and M752, respectively.